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# EFFICACY OF COMBINATION OF SYSTEMIC AND NON-SYSTEMIC FUNGICIDES AGAINST STEM ROT OF RICE

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**ABSTRACT**
Stem rot of rice, caused by *Sclerotia oryzae* is a serious threat to rice production in North India. Fungicides only provide limited control of this pathogen but also have ill effects on the environment. In an attempt to develop better integrated strategies for management of this disease, *in vitro* experiments were conducted to find out the compatibility of different systemic and non systemic fungicides with *Sclerotia oryzae* and fungal and bacterial biocontrol agents. Among the five systemic fungicides tested, Contaf was highly effective in reducing mycelial growth of *S. oryzae* at low concentration. Of the four non-systemic (contact) fungicides Chlorothalonil was highly effective against the pathogen *in vitro*. *Trichoderma* and *Pseudomonas* isolates showed variable responses against the tested fungicides *in vitro*. The results indicated that Bavistin was comparatively less inhibitory to the bioagents and it also gave satisfactory inhibition to the pathogen growth. So the combination of Bavistin at low concentration with bioagents increased the efficacy of both, subsequently reducing the chances of development of resistant pathogenic strains. More detailed studies are required to elucidate formulations of antagonists and alternative fungicides for more successful protection against the disease.

## INTRODUCTION

Stem rot caused by *Sclerotium oryzae* is one of the major diseases of rice in India. It is prevalent in Punjab and Tamilnadu and has been observed to be severe in ill drained soil in Eastern Uttar Pradesh and adjoining districts of Bihar and also in some parts of Orissa and Madhya Pradesh for years. Little information is available on the efficacy of fungicides in the control of stem rot of rice. It was considered desirable to evaluate the efficacy of some chemical fungicides against the disease. Efficacy of bacterial biocontrol antagonists has been reported by Elangovan and Gnanamanickam (1992); and Sakthivel *et al.* (1988) . But biocontrol agents alone cannot manage the disease completely when infection is already established in huge amounts in the field. Therefore, farmers favoured fungicides for managing the disease. Fungicides are deleterious to the environment and also harmful for the soil productivity and human and animal health. Due to the disadvantages of fungicides, integrated disease management programs are applied, in which judicious and recommended use of fungicides and their integration with biocontrol agents is favoured. Since fungicides may have deleterious effect on the pathogen as well as the antagonists, an understanding of the effect of fungicides on the pathogen and antagonists, would provide information on the selection of selective fungicides and fungicides resistant antagonists for compatibility studies. The idea of combining Bio Control Agent (BCA) with fungicide is for the development or establishment of desired microbes in rhizosphere (Papavizas and Lewis, 1981). Further the antagonism of BCA was influenced by the addition of fungicides (Kay and Stewart, 1994; Naar and Kecskes, 1998). Hence studies were undertaken to test the compatibility of different fungicides with Bio Control Agent (BCA).

## MATERIALS AND METHODS

Efficacy of different systemic and non systemic fungicides at different concentrations was evaluated on radial growth of test fungus by Poisoned Food Technique. 100mL stock solution (1000$\mu$g/mL) of each fungicide was prepared in sterilized distilled water in 500mL Erlenmeyer flask. Required amount of stock solution was poured into 150 mL Erlenmeyer flask containing 100mL of sterilized melted PDA so as to get final concentrations of 2.5, 5.0, 10.0 and 20.0$\mu$g/mL for systemic fungicides and 25, 50, 100 and 200$\mu$g/mL for non-systemic fungicides. A formula C1V1 = C2V2 was used to find out the amount of stock solution to be added to PDA to get above concentrations, where C1 and C2 are concentration of stock solution ($\mu$g/mL), respectively, and V1 is the volume (mL) of stock solution to be added to the measured volume (V2) of PDA. Each Petri plate containing PDA of different concentration was centrally inoculated with 5 mm mycelial discs of *S. oryzae* and *Trichoderma* sp. (isolate 1) separately cut from the actively growing mycelium of the pathogen and biocontrol agent. Non-amended PDA plates served as check. Three replications were maintained for each treatment. Plates were incubated at 28 + 1ºC and colony diameter was measured when the check plates were fully covered with mycelial growth of test fungus. Per cent inhibition of the mycelium growth was calculated by using following formula:

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition, C = Radial growth in check in mm and T = Radial growth in treated plates in mm.

Bacterial suspension was prepared in sterilized distilled water in sterilized test tube. 150mL Erlenmeyer flask containing 100mL of sterilized King's B medium was melted and allowed to cool up to lukewarm temperature. 1.5mL bacterial suspension was poured into 150mL Erlenmeyer flask containing 100mL of sterilized melted King's B medium. Twenty mL of media mixed with bacterial suspension were poured in sterilized 90mm Petri plate under aseptic condition and allowed to solidify. Stock and other concentrations of systemic and non systemic fungicides were prepared as mentioned above. Sterilized paper disc (5mm) were dipped in chemical solution of different concentrations and placed on solidified medium. One Petri plate contained four sterilized paper disc. Plates were incubated at 28 + 1ºC and diameter of inhibition zone was measured and compared with the check after 48h of inoculation.

## RESULTS AND DISCUSSION

**Effect of systemic fungicides and non systemic fungicides on pathogen-** Data presented in Table 1 showed the effect of different concentrations of systemic fungicide namely Bavistin, Folicur, Tilt, Contaf and Beam on the radial growth of *S. oryzae*. Except Beam, most of systemic fungicides tested, showed significant inhibitory effect on the mycelial growth of *S. oryzae* as compared to check. Contaf was found highly effective in inhibition of mycelial growth of *S. oryzae* even at low concentration. Beam was found less effective in the inhibition of radial growth of *S. oryzae*. Bavistin also showed satisfactory mycelial inhibition of the pathogen.

In all the non systemic fungicides tested at different concentrations against the radial growth of *S. oryzae* (Table 2). Except Kocide all other non systemic fungicides showed inhibitory effect on the mycelial growth of *S. oryzae* as compared to check. Chlorothalonil was highly effective even at low concentration *i.e.* 25$\mu$g/mL. Kocide was found least effective in the inhibition of radial growth of *S. oryzae*. Similar result was found when Sharma and Mehrotra (1986) tested 17 fungicides against *S. oryzae (Magnaporthe salvinii)*, 5 were effective *in vitro* and of these Bavistin (Carbendazim) and Topsin-M (thiophanate methyl) controlled the disease and increased yield of potted plants. Kumar *et al*. (2011) also screened Bavistin and Thiram *in vitro* against *S. oryzae* and found reduced growth of the pathogen.

**Effect of Systemic and non- systemic fungicides on radial growth of *Trichoderma* sp. and bacterial biocontrol agent, *Pseudomonas*-** Table 3 and 4 shows sensitivity of *Trichoderma* spp isolate 1 to different fungicides. *Trichoderma* spp isolate 1 showed relatively less sensitivity to insensitivity against Mancozeb. On the other hand, it showed high sensitivity to fungicides like Chlorothalonil, Tilt, Contaf and Bavistin. Tilt, Contaf, and Bavistin at 5$\mu$g/mL concentration showed maximum inhibition of *Trichoderma i.e.* 90.00 to 95.55 per cent. However, Chlorothalonil at 100$\mu$g/mL showed maximum inhibition of 91.66 per cent. The result are confirmatory with the findings of Mishra (1998), who observed that Bavistin was more inhibitory against *G. virens, T. virens* and *T. harzianum* as compared to other fungicides. Insensitiveness of *Gliocladium virens* against captan, metalaxyl, and copper oxychloride have been reported by De and Mukhopadhyay (1990). Insensitiveness of *G. virens* to Vitavax, Captan and Thiram has been demonstrated by Mishra (1998). The benzimidazole groups of fungicides (carbendazim and benomyl) were toxic to *T. harzianum* and *T. longibrachiatum* even at 1$\mu$M concentration (Viji *et al.,* 1997). Sinha *et al*. (1983) demonstrated that Bavistin was highly inhibitory to *T. viride* at 1.25ppm. Khan and Shahzad (2007) also found that Topsin-M and Carbendazim were able to inhibit the growth of *Trichoderma* species even at low concentration. The differential response to biocontrol agents to various fungicides might be due to their inherent resistance to most of fungicides and their ability to degrade chemicals (Papavizas, 1985 and Viji *et al.,* 1997). Fungicides those are active against a narrow spectrum of plant pathogen but not against biocontrol agent offer an opportunity for integration of chemical and biocontrol agents. PCNB- resistant strains of *Trichoderma* have been used successfully for management of Southern stem blight of soybean caused by *Sclerotium rolfsii* (Singh and Uppadhyay, 2009). When biocides are applied in sublethal doses, some fungal biocontrol agents (*Trichoderma* sp. etc.) are known to proliferate and produce antibiotics in soil (Papavizas, 1985). Further, their application may metabolically weaken the pathogen and make it vulnerable to potent biocontrol agents.

Data in Table 5 and 6 show sensitivity of fluorescent *Pseudomonas* isolate against systemic as well as non systemic fungicides respectively. In case of systemic fungicides (Table 5) diameter of inhibition zone was maximum in case of Bavistin *i.e.* 2.66mm followed by Contaf (2.33mm), at 20$\mu$g/mL concentration. In case of non-systemic fungicide, diameter of inhibition zone was highest for Chlorothalonil (8.66 mm) followed by Mancozeb (7.00mm) (Table 6). Inhibition zone was not formed in case of Thiram. These *in vitro* studies clearly

**Table 1: Effect of systemic fungicides on radial growth of *S. oryzae* on PDA, at 28 ± 1ºC**

| Fungicides | Concentration ($\mu$g/mL) Radial growth* (mm) | | | | | Per cent inhibition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5.0 | 10.0 | 20.0 | Mean | 2.5 | 5.0 | 10.0 | 20.0 |
| Bavistin | 20.66 | 15.16 | 11.16 | 5.00 | 13.00 | 77.04 | 83.15 | 87.60 | 93.33 |
| Folicur | 31.16 | 13.83 | 10.67 | 5.00 | 15.41 | 65.37 | 84.63 | 88.14 | 93.33 |
| Tilt | 31.17 | 13.67 | 09.33 | 5.67 | 14.95 | 65.36 | 84.81 | 89.63 | 93.70 |
| Contaf | 07.33 | 5.00 | 06.00 | 5.00 | 5.58 | 91.85 | 93.33 | 93.33 | 93.33 |
| Beam | 74.00 | 67.50 | 71.00 | 74.00 | 68.20 | 32.96 | 25.27 | 21.11 | 17.77 |
| Check | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | - | - | - | - |
| CD at 1% | 2.70 | | | | | | | | |

**Table 2: Effect of non systemic fungicides on radial growth of *S. oryzae* on PDA, at 28 ± 1ºC**

| Fungicides | Concentration (µg/mL) Radial growth* (mm) | | | | | Per cent inhibition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | Mean | 25 | 50 | 100 | 200 |
| Thiram | 30.33 | 24.00 | 12.00 | 5.00 | 17.83 | 66.30 | 73.33 | 86.66 | 93.33 |
| Mancozeb | 43.00 | 39.66 | 33.66 | 15.00 | 32.83 | 52.22 | 55.93 | 62.60 | 83.33 |
| Captan | 75.00 | 65.66 | 42.33 | 31.66 | 53.66 | 16.66 | 27.04 | 52.96 | 64.82 |
| Kocide | 90.00 | 90.00 | 85.33 | 80.00 | 86.25 | 00.00 | 00.00 | 02.96 | 11.11 |
| Chlorothalonil | 12.00 | 5.00 | 5.00 | 5.00 | 6.75 | 85.55 | 93.33 | 93.33 | 93.33 |
| Check | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | 00.00 | 00.00 | 00.00 | 00.00 |
| CD at 1% | 1.70 | | | | | | | | |

**Table 3: Effect of systemic fungicides on radial growth of *Trichoderma* sp. on PDA, at 28 + 1ºC**

| Fungicides | Concentration (µg/mL) Radial growth* (mm) | | | | | Per cent inhibition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5.0 | 10.0 | 20.0 | Mean | 2.5 | 5.0 | 10.0 | 20.0 |
| Tilt | 7.5 | 5.0 | 5.0 | 5.0 | 5.62 | 91.66 | 94.44 | 94.44 | 94.44 |
| Contaf | 11.0 | 7.5 | 7.16 | 7.0 | 8.16 | 87.77 | 91.66 | 92.04 | 92.22 |
| Bavistin | 10.33 | 4.0 | 4.0 | 4.0 | 5.58 | 88.52 | 95.55 | 95.55 | 95.55 |
| Check | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | - | - | - | - |
| CD at 1% | 1.113 | | | | | | | | |

**Table 4: Effect of non-systemic fungicides on radial growth of *Trichoderma* sp. on PDA, at 28 + 1ºC**

| Fungicides | Concentration (µg/mL) Radial growth* (mm) | | | | | Per cent inhibition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | Mean | 25 | 50 | 100 | 200 |
| Thiram | 60.33 | 57.66 | 19.00 | 15.66 | 38.16 | 32.96 | 35.93 | 78.88 | 82.60 |
| Mancozeb | 90.00 | 90.00 | 78.00 | 64.66 | 80.66 | 00.00 | 00.00 | 13.33 | 28.15 |
| Chlorothalonil | 17.83 | 11.16 | 7.50 | 8.33 | 11.20 | 80.18 | 87.60 | 91.66 | 90.74 |
| Check | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | - | - | - | - |
| CD at 1% | 5.04 | | | | | | | | |

**Table 5: Diameter of inhibition zone (mm) produced by systemic fungicides with bacterial bioagents**

| Treatment | Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|
| | 2.5 | 5.0 | 10.0 | 20.0 | Mean |
| Bavistin | 1.00 | 1.00 | 1.00 | 2.66 | 1.41 |
| Contaf | 1.00 | 1.00 | 1.66 | 2.33 | 1.50 |
| Check | - | - | - | - | - |
| CD at 1% | 0.68 | | | | |

**Table 6: Diameter of inhibition zone (mm) produced by Non systemic fungicides with bacterial bioagent**

| Fungicides | Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | Mean |
| Thiram | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mancozeb | 1.66 | 2.33 | 5.66 | 7.00 | 4.16 |
| Chlorothalonil | 4.66 | 6.66 | 8.00 | 8.66 | 7.00 |
| Check | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CD at 1% | 0.92 | | | | |

established that bacterial antagonists were able to tolerate different concentrations of fungicides. This supports the earlier findings of Vidyashekharan and Muthamilan (1995) that seed treatment fungicides Thiram and carbendazim were not inhibitory to *Pseudomonas fluorescens*. Lindaw *et al*. (1996) reported that combinations of *Pseudomonas fluorescens* with antibiotics were effective in control of fire blight and frost injury to pear. Recently, Kader *et al*. (2012) found that use of combinations of fungicides and fungicide tolerant fungal and bacterial biocontrol agents were effective in controlling root rot of vegetables in pot conditions. However, so far not so many attempts have been made on the combined efficacy of *P. fluorescens* with fungicides against plant diseases, while few reports were available on the effects of pesticides on plant diseases suppressing bacteria other than Pseudomonads (Heydari *et al.*, 1997; Sheela and Venkitashan, 1977; Chenzhiyi *et al.*, 1998).

On the basis of present *in vitro* experiments it can be concluded that at low level Bavistin and Thiram both are compatible with biocontrol agents and can be used in combination with biocontrol agents for the management of stem rot disease of rice. Further, glass house and field studies should to conducted to determine the potential of biocontrol agents tolerating small amount of fungicides against *S.oryzae*. Moreover, the use of combination of fungicide tolerant biological control agents with reduced levels of fungicide in IPM programme would result in disease suppression similar to that achieved with full dosage of fungicides (Monte, 2004).

## REFERENCES

**Chenzhiyi, L. G., Zhao, T., Yan, D. and Wan, H. 1998.** Effect of fungicides on antagonistic bacteria from rice seed surface. *Chinese J. Biol. Contr.* **14:** 72-74.

**De, R. K. and Mukhopadhyay, A. N.1990.** Control of Damping off in tomato by seed coating with Gliocladium virens. Proc. Int. Conf. Seed. Sci. Technol., New Delhi. pp. 120.

**Elangovan, C. and Gnanamanickam, S. S. 1992.** Incidence of *Pseudomonas fluorescens* in rhizosphere of rice and their antagonism towards *Sclerotium oryzae*. *Indian Phytopathol*. **45:** 358-361.

**Heydari, A., Mivaghi, I. J. and Mc Closkey, W. B. 1997.** Effect of three soil applied herbicides on populations of plant disease suppressing bacteria in cotton rhizosphere. *Pl. Soil*. **195**:75-81.

**Kader, M. M. A., Mougy, N. S. E., Aly, M. D. E., Lashin, S. M. and Mohamady, R. S. E. 2012**. Soil drench with fungicides alternatives against root rot incidence of some vegetables under greenhouse conditions. *Int. J. Agri. Forestry*. **2**: 61-69.

**Kay, S. J. and Stewart, A. 1994.** The effect of fungicides on fungal antagonists on white rot and selection of dicarboxamide-resistant biotypes. *Plant Path.* **43**: 863-871.

**Khan, M. O. and Shahzad, S. 2007.** Screening of *Trichoderma* species for tolerance to fungicides. *Pak. J. Bot.* **39**: 945-951.

**Kumar, A., Singh, R. and Jalali, B. L. 2011.** Variability in growth and biochemical components of *Sclerotium oryzae* populations of rice and its relevance to virulence. *J. Mycol. Pl. Pathol.* **41**: 143-145.

**Lindaw, S. E., McGourty, G. and Elkins, R. 1996.** Integration of antibiotics with *Pseudomonas fluorescens* and frost injury to pear. *Phytopathology*. **86**: 841-848.

**Mishra, D. S. 1998**. Comparative efficacy of some biocontrol agents against *R.solanii* kuhn, the cause of sheath blight of rice. M.Sc. thesis G.B. Pant Univ. of Agriculture and Technology, Pantnagar.

**Monte, E. 2004.** Understanding *Trichoderma*: between biotechnology and microbial ecology. *Int. Microbiol.* **4**: 1 - 4.

**Naar, Z. and Kecskes, M. 1998.** Antagonsits of Trichoderma atroviridae and Trichoderma viride against *Sclerotinia minor* as influenced by Mancozeb, Benomyl and Vinclozolin. *Acta. Phytopathologica et Entomologica Hungarica*. **33**: 123-130.

**Papavizas, G. C. and Lewis, J. A. 1981.** Introduction and augmentation of microbial antagonists for the control of soil borne plant pathogens. In: Biological Control in Crop Production, G.C. Papavizas (Ed.), Allanheld Osmum, New York. pp. 305-322.

**Papavizas, G. C.1985.** *Trichoderma* and *Gliocladium:* biology, ecology and potential of biocontrol. *Annu. Rev. Phytopathol.* **23:** 23-54.

**Sakthivel, N., Sivamani, Anuratha, C. S., Savithiry, S., Gnanamanickam, S. S., Gnanamanickam, S. S. and Mahadevar, A. 1988.** Beneficial bacteria for plant disease management. Advances in research on plant pathogenic bacteria based on the proceedings of the National Symposium on Phytobacteriology held at the University of Madras, Madras, India during March 14-15, 1986. 213-220.

**Sharma, S. K., Mehrotra, R. S. 1986.** Survival of rice stem rot pathogen in soil. *Geobios.* **13**: 97-99.

**Sheela, M. S. and Venkiteshan, T. S. 1997.** In vitro evaluation of selective fungicides on the nematode pathogenic bacteria, *Bacillus circulans. Pest Manage. Horti. Eco.* **3**: 49-51.

**Singh, B. K. and Upadhyay, R. S. 2009.** Management of southern stem blight of soybean by PCNB- resistant mutants of *Trichoderma harzianum* 4572 incited by *Sclerotium rolfsii*. *J. Agri. Technol.* **5**: 85-98.

**Sinha, A. P., Agnihotri, V. P. and Singh, K.1983.** *In vitro* effect of carbendazim on the growth and sporulation of some common soil fungus. *J. Mycol. Pl. Pathol.* **13:** 350-351.

**Suparyono, S. 2003.** Evaluation of antagonistics bacteria for biological control agent of rice sheath blight and stem rot. *Penelitian Pertanian Tanaman Pangan* (Indonesia). **19:** 1-6.

**Vidyashekharan, P. and Muthamilan, M. 1996.** Development of formulations of *Pseudomonas fluorescens* for the control of chick pea wilt. *Plant Dis.* **79**: 782-786.

**Viji, G., Manibhushanrao and Baby, U. I. 1997.** Non target effect of systemic fungicides on antagonistic microflora of *Rhizoctonia solanii*. *Indian Phytopath.* **50**: 324-328.